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Wilt and Cold Resistance of Self-Fertilized Lines of Alfalfas

GEORGE L. PELTIER AND H. M. TYSDAL

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SUMMARY

The relative reactions to cold and wilt of self-fertilized lines of alfalfa through to the fifth generation are reported.

Some of the reasons for undertaking a breeding program are given as well as methods used in the controlled cold and wilt determinations. Preliminary studies showed that reinoculating healthy individuals which had already resisted one wilt infection test served to eliminate additional plants, but the percentage healthy was much higher after the reinoculation than after the first inoculation.

The foundation materials from which selections were made consisted of selected plants from a number of old Nebraska fields and a large collection of seed lots assembled by H. L. Westover from many of the alfalfa-growing regions of the world. Some plants from the old fields yielded seed, the seedlings from which had a high degree of resistance to both cold and wilt, whereas others proved to be quite susceptible to either wilt or cold or to both. The latter were usually promptly discarded.

The results obtained in the selfing program may be summarized as follows:

(a) Selfing without elimination or with cold elimination only, resulted in decreasing wilt resistance with advancing generations. Wilt elimination, within self-fertilized lines, resulted in maintaining the wilt resistance of originally highly resistant parents, or of increasing the wilt resistance of originally moderately resistant parents.

(b) There is a marked inheritance of wilt resistance, as evidenced by the fact that higher resistance among parental groups resulted in higher resistance of the progeny. The parental group having the highest resistance produced progeny with the greatest uniformity with respect to wilt resistance. The advanced selfed generations bred truer for wilt resistance, the fifth being much more uniform than the first or second generation.

(c) Some lines were consistently high in wilt resistance.

(d) High wilt and cold resistance do occur in the same individuals, but the results point to independent segregation. More than one factor, possibly three, are involved in the inheritance of wilt resistance. Data so far obtained also indicate that more than one factor are involved in the inheritance of cold resistance.

(e) In general, the reaction of lines to cold resistance upon selfing has shown the same behavior as the wilt studies already mentioned. Rigid elimination by cold within self-fertilized lines has served to maintain cold resistance, although selfing without elimination tends to markedly decrease cold resistance.

(f) Preliminary results of compositing seed from desirable plants or "strain building" indicate promising possibilities by this method.

Wilt and Cold Resistance of Self-Fertilized Lines of Alfalfas

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A winterhardy and wilt-resistant alfalfa is necessary for the continued success of alfalfa culture in large areas of the middle west. After the disastrous winter of 1917-18, the alfalfa acreage in Nebraska dropped 200,000 acres, primarily because of winterkilling. From 1918 a slow increase in acreage occurred until 1924, when 1,358,000 acres were grown. A gradual decrease in acreage has since occurred until approximately 1,110,000 acres of alfalfa were grown in 1933. A number of factors have been responsible for this decrease of about one-quarter million acres, one of which is bacterial wilt of alfalfa caused by *Aplanobacter insidiosum* L. McC. Thus winterkilling, together with the ravages of wilt, has threatened the stability of alfalfa production, not only in Nebraska but also in neighboring states.

In the Platte valley of Nebraska, where wilt conditions have been severe over a period of years, Grimm and common alfalfa are usually plowed up within three to five years after sowing because of severe reductions in stand. On the other hand Hardistan and Turkistan, which are more wilt resistant, maintain a stand for seven years or more. A corresponding difference appears to hold under upland conditions, although productive stands remain somewhat longer.

Assuming that the acreage of the whole state would have an average life of seven years with a wilt-resistant alfalfa, one-seventh of the acreage, or 125,700 acres (omitting the 20 per cent which is turned under in short rotations), would have to be plowed up and replanted each year to maintain the total acreage. If wilt takes the stand out two years earlier, making the length of stand five years instead of seven, one-fifth or 176,000 acres must be plowed up and replanted each year. The difference amounts to 50,300 acres, not allowing for any failures in planting. It would cost approximately $200,000 annually to prepare and seed these 50,300 acres without considering the loss of the crop itself. This cost could be avoided if a productive alfalfa were grown which would last only two years longer than the alfalfas now commonly available.

Breeding for resistance to disease and for other desirable characters is now an established practice in many crop-improvement programs and is yielding distinct and valuable results. Since various cultural treatments have afforded little effective control of the bacterial wilt disease of alfalfa and since in addition a cursory study of alfalfa-plant populations shows a wide variability of type in any given field, and a wide divergence of adaptability from field to field due to origin of seed, a systematic effort for the improvement of alfalfa was initiated. A preliminary study led to

[1] Co-operative investigations between the Departments of Agronomy and Plant Pathology, Nebraska Agricultural Experiment Station, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

the finding of wilt-resistant plants in certain old fields in Nebraska, and from this it was concluded that strains of Turkistan origin were more or less wilt-resistant. Turkistan, however, is not desirable in many of its characters, particularly for regions east of the Mississippi river. It was the aim, therefore, to develop strains having high cold and wilt resistance and other desirable characters such as vigor of growth and high seed production.

To this end an inbreeding program was begun in 1928, and the results reported in this paper deal with the behavior of inbred lines of alfalfa with respect to cold and wilt resistance. An effort was made to discover the reaction of individual plants to both cold and wilt inasmuch as the two have been intimately associated under certain field conditions. While a limited amount of hybridization and "strain building" has been attempted, the main emphasis to date has been placed upon an effort to produce lines more or less homozygous for cold and wilt resistance.

TECHNIQUE OF TESTING

WILT RESISTANCE

The wilt determinations were made as follows: 100 plants or their equivalent from each seed lot to be tested were grown for one month or longer in the greenhouse, inoculated in either of two ways, depending on the size of the plants, and then transplanted to the field or greenhouse beds. Several rapidly growing two-weeks-old test-tube cultures of the organism from different sources were placed in water at the rate of one culture tube per liter and the bacterial mass washed from the medium. The roots of the larger plants were lightly scraped near the crown with a sharp scalpel while in the bacterial suspension. In case of the smaller plants the ends of the roots were severed under the suspension with a sharp razor blade upon a block of wood. The latter method was preferred, since it was not only more rapid but the plants could be more uniformly inoculated.

Conditions as nearly optimum as possible for the development of the disease were supplied, particularly with respect to moisture. At the end of about five months all plants were lifted and the percentage and severity of infection determined from top and root symptoms.

The plants were classified as healthy, slightly infected, moderately to severely infected, and dead. The total percentage of infection at the conclusion of the experiments was based on the number of plants alive one month after transplanting, since some plants failed to establish themselves. All figures reported in this paper dealing with wilt resistance are given as the percentage of healthy or non-infected plants recorded at the conclusion of any test.

COLD RESISTANCE

The method of determining hardiness in seedling alfalfas consisted essentially in the following general procedure: Each seed lot was planted in three rows in a cypress flat (15x18x6 inches) with a control alfalfa of known hardiness in alternate rows and allowed to grow under optimum conditions in the greenhouse for one month. The flats were then trans-

TABLE 1.—*The wilt resistance of the control varieties compared to reinoculated healthy plants of Hardistan which had survived one wilt test*

Kind of alfalfa	Date and location of tests	Replications	Total plants	Healthy plants	Infected plants	
					Slight	Moderate to severe
		No.	*No.*[1]	*P. ct.*	*P. ct.*	*P. ct.*
Grimm (controls)	1932, greenhouse	8	439	0	9	91
Hardistan (controls)	1932, greenhouse	7	285	41	5	54
Hardistan (reinoculated	1933, field	7	84	86	10	4
Hardistan (controls)	1933, field	8	333	41	21	38
Grimm (controls)	1933, field	9	387	4	10	86

[1] The percentage of infection is based on the number of growing plants present one month after inoculating and transplanting in the field or greenhouse.

ferred to the hardening chamber and held at a temperature of 2° to 4° C. for two weeks. Before the seedlings were frozen the soil was brought to a high and uniform moisture content. The seedlings were then exposed in the freezer room for a number of hours (8 to 24) to a temperature at some point between -10° and -20° C. The length of exposure was gauged so that approximately 50 per cent of the control alfalfa survived. After this freezing, the seedlings were removed to the greenhouse and two weeks later survival counts were made. Usually nine or more rows of each seed lot were tested. The actual percentage of survival of the alfalfas under test was calculated in terms of the control and comparisons between seed lots were made by this standard.

Occasionally plants which survived a wilt-infection test were planted in soil in the flats, allowed to grow for a month or so in the greenhouse, hardened off for a two-weeks period, and then exposed to low temperatures.

STUDIES OF METHODS

EFFECT OF REINOCULATION ON SURVIVORS OF ONE INFECTION TEST

In the spring of 1933 all healthy plants of the more promising selections which came through the wilt-infection tests in the greenhouse were reinoculated and transplanted in the field. Two objectives were in mind: (1) to eliminate further the susceptible population and (2) to determine the reliability of a single test. In Table 1 are listed the results obtained with the two varieties employed throughout the investigations as standards or controls, namely Grimm, highly susceptible, and Hardistan, moderately resistant to wilt. As can be seen from the controls, the greenhouse and field inoculations are quite comparable.

Of the Hardistan survivors from the greenhouse tests which were reinoculated and transplanted in the field in May, 86 per cent remained healthy, and only 4 per cent were found to be moderately to severely infected in October. In other words, the first inoculation eliminated the great majority of the highly susceptible individuals although a few, probably escaping the first test, were eliminated, together with a small percentage of relatively resistant plants which became slightly infected.

Similar results were obtained on reinoculating a number of the selections (Table 2). In the main the same conditions held, irrespective of the type of selection or generation involved. It is of interest to note that among the 155 comparisons only 10 reversals of varying ranges occurred; i. e., a higher percentage of infection was obtained on reinoculating the survivors of the first test.

TABLE 2.—*The effect of reinoculating healthy individuals of a number of alfalfa selections which had survived one wilt test*

Source of selections	Selections involved	Generation	Comparisons	Average healthy	
				First test	Reinoculation
	Number	*Number*	*Number*	*Per cent*	*Per cent*
Nebraska (old fields)	10	1	15	63	87
	10	2	15	60	88
	10	3	10	47	78
	10	4	15	56	80
Turkistan	29	1	69	52	84
	5	2	7	46	80
Persia	3	1	9	56	80
Manchuria	1	1	3	23	63
Chile	3	1	12	33	62

A number of attempts have been made to reinoculate the few remaining healthy Grimm plants which have survived the wilt tests the past four years. In all instances, however, these have succumbed to a second test. It is quite apparent, therefore, that Grimm is homogenous so far as its susceptibility to wilt is concerned.

In order to determine more closely what actually happens on reinoculation, an analysis was made of the 69 comparisons of S_1 Turkistan selections. This analysis is given in Table 3. The data show that the few plants remaining from the susceptible lines after the first test were not as resistant as those survivors from the resistant lines. Those eliminated by the second inoculation usually exhibited slight rather than severe wilt symptoms.

Thus further elimination of susceptible individuals can be obtained by inoculating survivors a second time, thereby insuring the elimination of a higher percentage of susceptible individuals and decreasing the population of selections to be selfed. Of more importance, however, is the fact that in all the wilt tests resistance of any selection based on one inoculation

TABLE 3.—*An analysis of 69 comparisons by groups of the effect of reinoculation of S_1 Turkistan selections*

Lines grouped according to following classes	Average healthy first inoculation	Average healthy reinoculation	Comparisons
Per cent healthy	*Per cent*	*Per cent*	*Number*
20 to 39	26	74	16
40 to 49	45	71	14
50 to 59	54	86	15
60 to 69	63	89	13
70 to 100	79	90	11

must be considered solely as relative and not absolute, since under proper environmental conditions some survivors may be infected by a second inoculation.

EFFECT OF SEED SOURCE ON THE WILT RESISTANCE OF OPEN-POLLINATED SEED

An opportunity was afforded to make a direct comparison of the resistance of plants from open-pollinated seed of the same individuals, grown at two different locations, through the use of cuttings. A brief description of the propagation of alfalfa by cuttings is herewith included since this method may have certain advantages in an alfalfa crop improvement program.

Two types of cuttings were propagated, namely, crown and stem cuttings. The greatest number of crown cuttings can be obtained from field-grown plants in the late fall or early spring when the greatest number of crown buds are present. As many as 200 cuttings were made from one plant. The main consideration in making the cuttings was to include a small bit of the root cambium. The percentage of cuttings which were successfully rooted varied with the individual plant and to some extent with the variety or strain of alfalfa. On the average, better than 50 per cent of the cuttings propagated from alfalfa crowns took root.

When it was desired to retain the original plant, stem cuttings were resorted to. After the top growth was a foot high or more, the stems were cut into sections so that rooting took place at one node and top growth occurred at the second. In other words, a cutting could be made from every two nodes from the base of the stem to the terminal bud. The percentage of cuttings that rooted was smaller than with crown cuttings and averaged somewhat below 50 per cent. Here again it was found that cuttings from individual plants and varieties or strains of alfalfa varied greatly in their ability to root successfully.

It can be readily seen that a large series of cuttings can be obtained from a single desirable plant in the course of a year, under greenhouse conditions. The increase of plants through the propagation of cuttings has merit since a large population can be increased within a short time.

During the dry summer of 1931, a seed crop from the selections grown at Lincoln was obtained. In 16 comparisons between seed grown at Lincoln and at North Platte in 1931, it was found that the seedlings of the same selections reacted about the same to wilt. Thus, seed raised at Lincoln and at North Platte of the same selections was composited for further testing for both cold and wilt resistance.

EFFECT OF SEED-CROP YEAR ON THE WILT RESISTANCE OF OPEN-POLLINATED SEED

Open-pollinated seed was obtained from a number of the selections growing at North Platte during the years 1930 and 1931. In some instances seed was raised from the same selections in both years. Seedlings from this seed were tested under the same conditions for wilt resistance. In comparing the results it was found that there was no material difference in the percentage of healthy plants of seed from the two crop years of the same selections. Therefore in further tests of wilt and cold resistance, seed from the same individuals raised in different years was composited.

SELECTION FOR WILT AND COLD RESISTANCE

SOURCE OF SELECTIONS

With the inception of the alfalfa-wilt investigations in 1927, a survey of the alfalfa fields in Nebraska and particularly those in the Platte valley was made to locate old fields which might have withstood the ravages of bacterial wilt as well as winter injury. A number of such fields were found. A brief history of the individual fields follows.

Fields 1, 5, and 6 were located in Buffalo and Dawson counties and were planted about 1911 with seed purchased as Turkistan. From the characteristic growth habits of the plants in these fields, they appear to have had a similar origin. All of them were plowed up in 1930-31.

Field 8 was planted in 1926 with seed obtained from an older field seeded with Turkistan in 1911, with perhaps the same origin as the above-mentioned fields. Field 8 still (1934) has a good stand and is fairly productive.

Field 3 was sown to Turkistan alfalfa in 1908. The growth habits of the plants differ from those in any of the other old fields. It is still fairly productive, although common alfalfa has been sown around this old planting for the third time since 1908.

Fields 4 and 9 were sown about 1902 and 1903 and the plants resemble Turkistan alfalfa. These plantings may have been the first seedings of Turkistan alfalfa in the Platte valley. Field 9 was plowed up in 1929, but Field 4 has a good stand at the present time and is fairly productive.

Field 7 was sown in 1918 with seed probably of Turkistan origin. It has a good stand and is productive.

Seed obtained directly from these fields, or by increasing seed from individual plants from these fields, has in the main been found to be resistant to both wilt and cold and has served as foundation stock for much of the selection work.

Three old fields (Nos. 12, 15, and 17) in Dawson county, two of which were planted as early as 1893, proved to be quite susceptible to wilt when seed from individual plants was tested for wilt susceptibility. None of these fields was of Turkistan origin, since Turkistan seed was not introduced into the United States until 1898. Likewise seed from selected plants in three fields (Nos. 13, 14, and 16) ranging from 10 to 16 years old in western Nebraska and probably common alfalfa, proved to be undesirable because of wilt susceptibility. Because the prevailing environmental conditions are unfavorable for the spread and rapid development of the disease, wilt is not a primary factor in decreasing stands in this area.

Individual plant selections were also made in plats at Lincoln, seeded in 1922. Three outstanding sorts, all of Turkistan origin, were found to be resistant to wilt, namely Hardistan, Turkistan (Com. No. 2230), and Provence (F. P. I. No. 34886). Seed of Hardistan alfalfa came originally from Field 1, previously described. Seed and individual plant selections of Ladak (F. C. I. No. 14135) completed the Nebraska material which was used as a basis for the selection work.

Supplementing this material was the large collection of seed lots assembled by Mr. H. L. Westover, Senior Agronomist, Division of Forage Crops and Diseases, U. S. Department of Agriculture, from many of the alfalfa-growing regions of the world. Most of these seed lots have been

under test for wilt and cold resistance since 1930 and numerous plant selections have been obtained through elimination by wilt and cold.

WILT AND COLD RESISTANCE OF ORIGINAL SELECTIONS

Mass selections.—In February of 1928, cuttings from a variable number of selected plants chosen from seven old fields in the Platte valley, together with plants from two alfalfa plats at Lincoln, were made, rooted in sand, potted, and later planted in rows in the field at the Substation at North Platte. This group of plants remained in the field at North Platte from the spring of 1928 until the fall of 1931, when they were discarded. In Table 4 is listed the percentage of field survival at the end of this period,

TABLE 4.—*The field survival (1928-31) of cuttings of mass selections from old fields compared with the resistance of seedlings to wilt and cold from open-pollinated seed of these cuttings*

Origin	Selection	Field survival of cuttings	Wilt resistance[1] of seedlings healthy [1]	Cold resistance of seedlings [2]
	Number	*Per cent*	*Per cent*	
Field No. 1	1-01	97	43 (5)[3]	99 (2)[3]
Turkistan (Com. No. 2230)[4]	2-01	67	50 (5)	107 (2)
Field No. 3	3-01	60	42 (6)	110 (3)
Field No. 4	4-01	100	73 (5)	111 (2)
Field No. 5	5-01	100	73 (5)	107 (3)
Field No. 6	6-01	87	63 (6)	121 (3)
Field No. 7	7-01	42	63 (5)	94 (3)
Field No. 8	8-01	40	76 (5)	107 (3)
Provence (F.P.I. No. 34886)[4]	10-01	60	80 (6)	112 (2)
Average		72	62	108

[1] Weighted average based on the number of plants in each test.
[2] Survival ratio to Turkistan F.C.I. No. 15754 as 100.
[3] Figure in parenthesis denotes number of trials.
[4] Seeded by the Agronomy Department at Lincoln in the spring of 1922.

together with the percentage of plants surviving the wilt and cold tests obtained from seed of the above-mentioned plants. It will be noted that the average field survival after four years is 10 per cent higher than for those in the infection tests, but this is to be expected since the field plants were not artificially infected with the wilt organism. The field survivors of the nine families ranged from 40 to 100 per cent, while in the infection tests the range was from 42 to 80 per cent. The relative rank of the percentage of survival under the two conditions was not the same. Both in the field and in the infection tests, however, all selections rated as high as, or higher, in wilt resistance than recent importations of Turkistan alfalfa. In cold resistance, likewise, they showed as much resistance as Turkistan (F. C. I. No. 15754) or more. Natural selection in the field over a period of 20 years or more may have contributed to the high cold and wilt resistance obtained.

Single plant selections.—During the month of November, 1928, a number of single plants were selected from seven old fields located in Buffalo and Dawson counties and from six fields in western Nebraska. Plants with

a characteristic type of growth, namely a large spreading top, a crown capable of producing offshoots, or the ability to reroot from the crown after the tap root had rotted, together with plants having an erect type of growth, were chosen. An occasional plant which had recovered from wilt, as observed by an examination of the roots, was also included. The majority of the plants therefore represented two distinct types although there were gradations between these types. In most instances the plants had a large number of fall buds, indicating that they were in a healthy and vigorous condition.

A number of cuttings were made from each plant, depending on the size and type of the crown and the number of fall buds and offshoots. Great variations in the percentage of rooted cuttings occurred, although an average of nearly 50 per cent was obtained from the entire lot of plants, the range in the percentage of rooted cuttings being from near 30 to over 80 per cent.

FIG. 1.—Cuttings (usually 25 in a block) from individual alfalfa plants screened with cheesecloth for selfing. Experiment Substation at North Platte, Nebraska, 1930.

The rooted cuttings from each individual plant were potted and carried through the winter in the greenhouse. In the latter part of April they were divided into two groups and planted in fields at Lincoln and North Platte, in blocks of 25 or less in order to facilitate screening for self-pollination later in the season (Fig. 1). Open-pollinated and selfed seed lots were obtained in 1930 at North Platte and in 1931 at both Lincoln and North Platte. All the plants within a family reacted similarly with respect to seed production and other characters. Detailed notes, including susceptibility to leaf spot and yellow blotch, were made in the field from 1929 until the spring of 1933.

In Table 5 are listed the percentages of surviving plants at Lincoln and North Platte, for a four-year period, together with the wilt and cold resistance of the seedlings from open-pollinated seed obtained in 1930 and 1931.

Omitted from the table because of their uniformly low survival both in the field and in the infection tests are single-plant selections and open-pollinated seed obtained from them from Fields 14 and 18, located in western Nebraska and Fields 15 and 17 in Dawson county. It is apparent in every instance that when a low field survival occurred, the plants from open-pollinated seed of these selections were also very susceptible to wilt in the infection tests. As found with the mass selections, the relative rank under the two conditions was not always the same, but the correlation between the original plants and their progeny was higher than shown in Table 4. As a rule, however, the fields survivals were higher than those of inoculated seedlings. There was no marked correlation between the cold resistance of the seedlings and the survival of plants in the field.

Cuttings of distinctive plants from a number of old fields yielded selections that withstood field conditions for a period of four years at Lincoln and North Platte. Likewise, open-pollinated seed obtained from some of the selections proved to be resistant to cold and wilt when their seedlings were tested. Even though some of the fields were 20 or more years old and the plants had endured a rigorous field test, apparently only those of Turkistan origin yielded selections that were resistant to both cold and wilt. The mere selection of outstanding plants that have withstood field conditions in wilt-infested localities for a long time does not mean that the seed from these plants will be resistant to cold, wilt, or both.

WILT RESISTANCE OF SELF-FERTILIZED LINES

Methods.—As already noted, a considerable amount of care had been exercised in choosing the original foundation stock for the breeding program. In some instances cuttings were made of original parent plants to facilitate greater seed production and for testing the original material. In other cases original seed lots were planted and the seedlings subjected to rigorous elimination. Seed has been raised both in the greenhouse and in the field, the large portion, however, having been produced under glass. When plants were to be self-fertilized in the field they were caged with cheese-cloth (Fig. 1). In the greenhouse individual plants were potted in seven-inch porous clay pots, the stems being supported by wire rings. Self-pollination was accomplished by rolling the flower racemes between the fingers with sufficient pressure to release the staminal columns. To avoid pollen contamination the hands were wiped with a cloth saturated with alcohol after pollination of each plant.

Good growth of the plants was obtained in the greenhouse and each year a large percentage of the plants yielded a gram or more of seed per plant. Since it takes from 500 to 900 seeds to weigh a gram, the amount was sufficient for testing and elimination purposes.

TABLE 5.—*The field survival (1929-33) of cuttings from single plant selections from old fields compared with the resistance of seedlings to wilt and cold from open-pollinated seed of these cuttings*

Origin	Plant	Selection	Field survival of cuttings		Wilt resistance of seedlings healthy [1]	Cold resistance of seedlings [2]
			at Lincoln	at No. Platte		
	Number	*Number*	*Per cent*	*Per cent*	*Per cent*	
Field No. 3	1	3-02	0	4	42 (3)[3]	126 (2)[3]
	2	3-03	40	70	35	.....
	3	3-04	100	93	40 (3)	.....
	4	3-05	96	98	80 (3)	104
	5	3-06	76	88	69 (2)	104
	6	3-07	100	100	80	125
	7	3-08	90	88	....	44
Field No. 4	1	4-02	84	80	70 (3)	105
	2	4-03	88	92	52 (4)	75 (2)
	3	4-04	80	84	72 (4)	112 (3)
	4	4-05	10	40	16	83
	5	4-06	92	96	67 (3)	73 (3)
	6	4-07	70	....	62	84 (2)
	7	4-09	100	....	84 (2)	112
	8	4-08	90	100	75 (2)	67
Field No. 5	1	5-02	92	94	71 (6)	108 (3)
	2	5-03	98	94	87 (6)	100 (3)
	3	5-04	47	0	13	....
	4	5-05	90	100	60 (5)	78 (2)
	5	5-06	80	100	68 (5)	57 (2)
	6	5-07	100	100	73 (3)	85
	7	5-08	90	92	58 (5)	77 (2)
Field No. 9	1	9-01	92	100	85 (4)	70 (2)
	2	9-02	100	100	73 (5)	78 (3)
	3	9-03	100	100	73 (5)	109 (3)
Field No. 12	1	12-01	0	4	12	45
	2	12-02	100	100	52 (3)	31
Field No. 13	1	13-01	0	13	13	....
	2	13-02	27	27	29	....
	3	13-03	95	88	39	....
	4	13-04	0	20	0	....
	5	13-05	0	7	0	....
Field No. 16	8	16-01	90	95	31 (5)	59 (3)

[1] Weighted average based on the number of plants in each trial.
[2] Survival ratio to Turkistan F.C.I. No. 15754 as 100.
[3] Figure in parenthesis denotes number of trials.

Throughout the whole program the object has been to eliminate the wilt- and cold-susceptible individuals in the self fertilized lines. Quite often the seed from an individual plant would be sown for a cold test, the surviving plants inoculated with the bacterial organism, and the ultimate survivors used to produce the succeeding generation of inbred seed. This is what is meant by cold and wilt elimination. Oftentimes space or amount

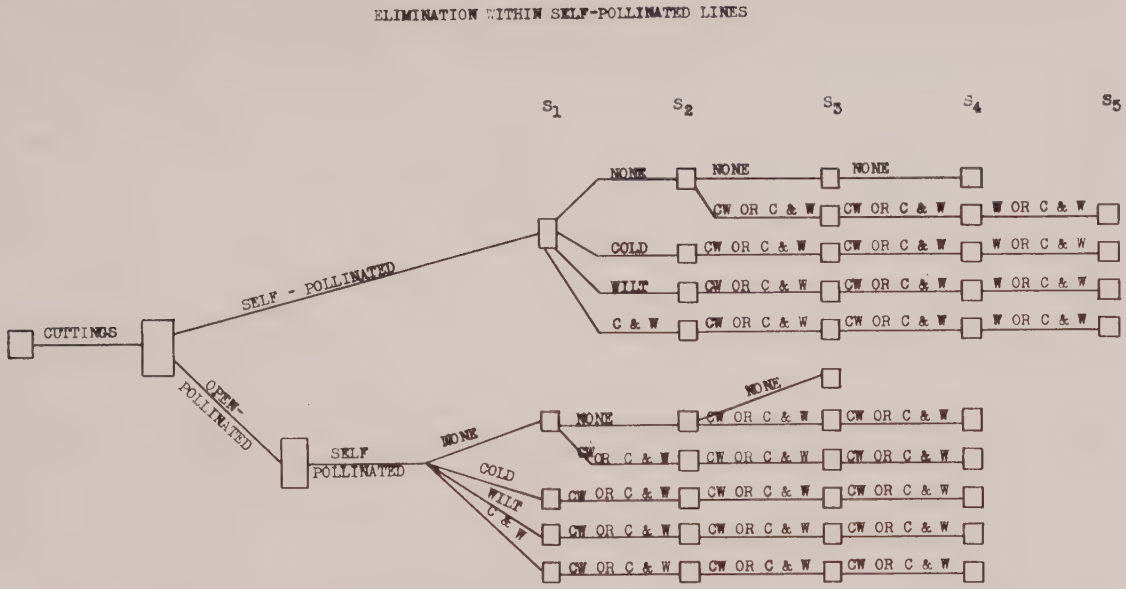

FIG. 2.—General procedure in the selfing program. Individual plant pedigrees were recorded and the chart presents the possible combinations of eliminations through which lines may have passed. All plants propagated as parental material were necessarily above the average in seed production, and were selected on the basis of vigor and other desirable characters. Many of the original plants were selected from fields 18 to 42 years old.

of seed limited the elimination in any single generation to cold alone or to wilt alone. There were therefore various types of elimination. To clarify the procedure as much as possible Figure 2 is used to show the various types of elimination involved.

Wilt resistance of non-selected, self-fertilized lines.—During the six-year period of this study the progeny of some of the original selected plants have been self-fertilized for five years and determinations of wilt

TABLE 6.—*Wilt resistance of alfalfa lines as affected by selfing without elimination*

Family	Percentage of healthy plants in generation as indicated				
	Open-pollinated	S_1	S_2	S_3	S_4
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1-01	43	64	....	....	....
2-01	50	24	14	....	....
	....	76	87	....	....
3-01	42	48	47	....	....
3-02	42	....	63	31	....
3-03	80	48	29	....	....
3-05	80	73	....	....	....
4-01	73	46	86	....	....
4-02	70	....	0	0	....
4-04	72	66	....	....	....
4-06	67	6	48	62	....
4-08	75	34	....	....	....
4-09	84	79	....	....	....
5-01	73	57	100	....	....
5-02	71	44	38	25	....
5-03	87	93	....	....	....
5-07	73	75	68	73	41 1 2 93 0 52
	....	....	43	....	52 80 7 17 37 28
6-01	63	77	....	....	....
7-01	63	16	86	....	....
8-01	76	59	....	....	....
9-01	85	65	57	....	....
9-02	73	61	....	....	....
Average	69	56	55	38	34

resistance have been made on some of the progeny in each generation. Table 6 gives the percentage of non-infected plants of the progeny of successive inbred generations in which no selection or elimination had been practised. Each figure represents a controlled wilt test on 100 seedlings, excepting a few instances where a smaller number of plants were used.

Although the trend in survivals as a whole is distinctly lower with each advancing generation, the reaction to selfing is by no means the same for all families: some of them showed an increase while others showed a decrease in wilt resistance. A good example can be seen in the fourth generation, where a large number of lines from the same family were tested. The segregation is striking, some lines showing almost 100 per cent resistance while others are completely susceptible.

Wilt resistance of selected self-fertilized lines.—The results of wilt tests on the self-fertilized lines whose population had been subjected to elimination in the preceding generation by cold, wilt, or both, are presented in Table 7. Individual plant pedigrees were kept which can be followed in the table by reading horizontally across the page. For example in Family 3-01, the dashes indicate that no tests were made until the third generation (87 CW). A second line from the same source (65 W), however, was tested in the second generation and three of its lines were tested (20 C, 38 C, and 63 C) in the third generation.

Even though the parents had survived a severe process of elimination, the progeny were not always high in wilt resistance. The general trend, however, was considerably different from that of the non-selected material and the average at the bottom of the table indicates no downward trend in wilt resistance through the fifth generation. While this average does not represent all families the same number of times, it probably gives a good indication of the trend. Examples can be found of both decreasing and increasing resistance with selfing, but perhaps the most interesting from the practical standpoint are those lines which maintained a high degree of wilt resistance throughout. Such lines can be found in Family 5-01, for example, where the original was 42 per cent, the first generation 90 per cent, and the second generation 86 and 98 per cent resistant. Likewise, one line in Family 5-07 was consistently high through the fifth generation and Families 8-02 and 9-02 had no progeny lower than 72 per cent resistance in any generation. In addition to these, it is also possible that lines which segregated for high resistance in advanced generations were as homozygous for wilt resistance as those which were consistently high from the beginning.

The effectiveness of the various types of elimination in wilt resistance as listed in Table 7 are summarized in Table 8, together with the resistance of the non-eliminated selections. It can be noted that elimination by cold did not improve the wilt resistance of the progeny very greatly, and not by any means to the same extent as elimination by wilt. Elimination by both wilt and cold has given about the same degree of resistance as by wilt alone.

TABLE 7.—*Wilt resistance of alfalfa lines as affected by selfing and method of elimination* [1]

Family	Percentage of healthy plants in generation as indicated					
	Open-pollinated	S_1	S_2	S_3	S_4	S_5
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Hardistan	46	36 CW 28 CW 45 CW 43 CW 42 CW 60 CW	35C			
1-01	43	25 CW 40 CW 61 CW	30 CW	32 CW 42 CW	95 W	
1-017	34	89 W				
2-01	50			67 CW		
3-01	42		29 C 50 C 63 W 38 CW	37 CW 20 C 38 C 63 C 23 C 27 C	76 W	
3-02	42	93 CW 96 CW 72 C 48 W	86 W 98 W 96 C 85 W 33 C 100 C	64 C 68 C 48 W 73 CW	72 C 48 C 48 C 30 C 60 C 37 C 61 C	96 CW 80 CW 83 W 69 CW
3-03	80			60 C 48 C		
3-0103	53	12 C 32 C 96 W CW	26 C 91 C	61 CW 83 CW	90C 67C	
3-09				91 CW 92 CW		
3-05	80	32 C 73 W 79 W	80 W			
3-06	69	67 W				
4-01	73	71 CW 47 CW 39 W 69 W 43 W	41 W 77 C 80 C 44 C 73 C 60 C 9 C	95 W 86 CW 88 CW		
4-02	70	87 C 52 W				
4-04	72		35 C 82 W	48 CW		
4-07	62	65 W 48 C 59 C 83 C 57 W	95 CW 59 CW 80 CW			
4-08	75	66 C 62 C	84 CW			

[1] The notations, C cold, W wilt, CW cold and wilt, indicate the elimination test to which the immediate parent plant had been subjected and which it had survived.

TABLE 7.—*Wilt resistance of alfalfa lines as affected by selfing and method of elimination* [1]*—(Continued)*

Family	Open-pollinated	Percentage of healthy plants in generation as indicated				
		S_1	S_2	S_3	S_4	S_5
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
		——	11 W 50 CW	32 C 77 C 84 W		
			78 C	93 CW		
5-01	73	100 W 73 W	47 C 80 C 69 W			
		65 W 81 C	50 C 67 C			
5-02	71	——	——	27 W	51 W	
5-03	87	71 W 84 W 86 W				
5-06	68	93 W				
				69 W	42 C	48 CW
				58 W	62 C 78 C 45 C 91 CW	90 CW 88 CW
		——	——	63 W	74 C 59 C	70 W 44 W 27 W
5-07	73			98 W		
				97 W	61 C 95 W	
		91 W				
5-08	58	75 CW				
5-09	68	65 C 56 C	80 CW			
6-01	63	—— 62 C	75 C 23 C 56 C	64 CW		
7-01	63	——	60 C 71 CW			
8-01	76	95 C 80 CW 100 CW				
		——	——	59 W	66 C	
9-01	85	——	100 CW			
		87 CW 72 W				
		——	80 W			
9-02	73	72 CW 90 CW 96 CW 93 CW	79 CW			
9-03	73	64 C 43 W 95 CW				
Average	65	67	63	62	64	70

[1] The notations, C=cold, W=wilt, CW=cold and wilt, indicate the elimination test to which the immediate parent plant had been subjected and which it had survived.

TABLE 8.—*Summary of wilt resistance of alfalfa lines as affected by selfing and methods of elimination*

Lines	Method of elimination			
	None	Cold	Wilt	Cold and wilt
Open-pollinated				
Number of lines	21	....	....	....
Percentage healthy	69	....	....	....
S_1 Number of lines	20	16	22	22
Percentage healthy	56	61	71	67
S_2 Number of lines	14	25	10	11
Percentage healthy	55	57	70	70
S_3 Number of lines	5	11	10	14
Percentage healthy	38	47	70	68
S_4 Number of lines	12	17	4	1
Percentage healthy	34	59	79	91
S_5 Number of lines	....	....	4	6
Percentage healthy	....	....	56	79
Weighted average				
Number of lines	51	69	50	54
Percentage healthy	49	57	70	70

In order to determine the influence of cold elimination on wilt resistance within the same generation 30 comparisons of nine families (Nebraska old fields) were studied. The seed of the selections was divided into two lots, one for the wilt tests and one for the cold determinations. The plants surviving the cold tests were inoculated and placed in the field, together with inoculated seedlings that had no cold elimination. The average percentage surviving only the wilt tests was 61, while those surviving the wilt after the cold test was 62 per cent. These results lead to the conclusion that elimination by cold in the same or preceding generation does not increase the wilt resistance of the remaining population to any appreciable extent.

The ability to transmit wilt resistance to self-fertilized progeny, regardless of the type of elimination, was calculated from the wilt tests of 409 strains (Table 9). The parental groups were divided into classes, each

TABLE 9.—*Wilt resistance and variability of selfed progenies grouped according to wilt resistance of their parents*

Parental groups, per cent healthy	Progeny tested	Average wilt resistance of progeny	Standard deviation
Per cent	*Number*	*Per cent*	
21- 40	55	55	23.7
41- 60	122	57	23.0
61- 80	191	66	20.7
81-100	41	76	12.1

of which had a range of 20 per cent in resistance, and the range of all classes was from 20 to 100 per cent. The wilt resistance of all the progeny coming from parent plants having a resistance between 21 and 40 per cent was averaged, and likewise each of the other groups. In the first group 55 progeny were tested and they averaged 55 per cent resistant. In the second group the wilt resistance of the progeny was 57 per cent, and with each succeeding group the wilt resistance was higher. Without exception it was found that the higher the wilt resistance of the parent, the higher the average wilt resistance of the progeny.

In addition to the wilt resistance, the variability of the resistance of the progeny in each classification as measured by the standard deviation is also given in Table 9. The variability was high in all groups except the one for which the parental group was very high in wilt resistance. This suggests that in groups intermediate in wilt resistance, segregation occurs resulting in both high and low wilt resistance, whereas when the parental resistance is exceptionally high there is less segregation, presumably because of the greater purity of the parents and the consequently lower variability of the progeny with respect to wilt resistance.

A matter of considerable interest from the standpoint of selection for wilt resistance is whether increased homozygosity has been attained in the advanced inbred generations. One way to determine this is to test the variability within succeeding inbred generations. To determine the variability in succeeding generations the progeny were grouped according to the wilt resistance of the parent and the standard deviations calculated on the

TABLE 10.—*Wilt resistance of selfed progenies grouped according to generations, and variability within generations grouped according to the wilt resistance of their parents*

Generation	Progeny tested	Average wilt resistance of progeny	Standard deviation
	Number	*Per cent*	
S_1	81	64	18.0
S_2	54	62	19.9
S_3	39	60	16.2
S_4	22	64	16.9
S_5	9	70	10.3

resulting frequencies. The results given in Table 10 show a decided reduction in variability after the second inbred generation and a particularly low variability in the fifth generation. While the fifth generation has the smallest number of progeny, it is also true that a single large deviation in this population would affect the standard deviation more than a similar deviation in a larger population. While too much emphasis is not to be placed on these results, the data suggest that increased homozygosity for wilt resistance has been obtained in advanced inbred generations.

COLD RESISTANCE OF SELF-FERTILIZED LINES

Cold resistence of non-selected self-fertilized lines.—Many of the inbred lines which have already been reported upon for wilt resistance were also tested for cold resistance by means of controlled freezing. The relative cold resistance of selfed lines without elimination is given in Table 11, following the same manner of presentation as for the wilt-resistance tests.

TABLE 11.—*Cold resistance of alfalfa lines as affected by selfing without elimination*

Family	Relative cold resistance (Turkistan check=100) in generation as indicated				
	Open-pollinated	S_1	S_2	S_3	S_4
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1-01	99	51	50		
2-01	107	82	82 104		
3-01	110	88	43 107		
3-02	126	....	65		
3-03	125	38	67	35	
3-06	109	22			
4-01	111	79			
4-02	105	....	....	35	
4-03	75	89			
4-04	112	169 104 73			
4-06	73	74	73		
4-07	84	112			
4-08	67	71			
4-09	112	13			
5-01	107	86			
5-02	108	....	97	29	
5-03	100	73			
5-07	85	....	103	48	49 38
6-01	121	99			
7-01	94	64			
8-01	107	62			
9-01	70	91	67		
9-02	78	60			
Average	99	76	78	37	44

The open-pollinated seed was in most instances high in cold resistance. With self-fertilization there was less cold resistance, as indicated by an average of all lines. Particular emphasis is laid upon the word "average", because there were exceptions which will have significance when the selected groups are discussed. The results tend to show that a gradual

reduction in cold resistance occurs in most instances with continued selfing and they parallel those already presented for wilt resistance.

Cold resistance of selected self-fertilized lines.—Table 12 shows the results of the determinations for cold resistance upon the seed of individual plants which had gone through the eliminations noted. Here again there were great differences in the reaction of some families as compared with others. On the whole, however, the trend was considerably different from that exhibited by the lines which had undergone no elimination. By appropriate elimination methods the cold resistance had been maintained at the original high level through the fifth generation. Some lines could be singled out for their continued high cold resistance, while others declined rapidly.

To determine the most effective method of elimination, Table 13 is presented, which summarizes the cold resistance of the lines which were not subjected to elimination, as compared to those which were eliminated by cold, wilt, or both. It can be noted that elimination by wilt served to markedly increase the cold resistance as compared with material not subjected to elimination. The lines subjected to cold averaged somewhat lower in cold resistance than those subjected to wilt but they were still considerably higher than those having had no elimination. This low average may be largely attributed to a group of lines in the third generation which were low in cold resistance. The lines having had both cold and wilt elimination maintained a high average and the resistance was about equal to that obtained with the original open-pollinated parents.

WILT AND COLD RESISTANCE OF COMPOSITES

As previously suggested, another method of attack on the general problem of improvement is by means of "strain building", i. e., intercrossing several selected plants and compositing the seed. An effort was made to include this possibility by artificially cross-pollinating selected plants produced from open-pollinated seed.

Crossing was attempted by tripping pollen from a plant onto a toothpick, which was then carried to another plant and the stigma allowed to snap on the accumulated pollen. Since all plants used were purple flowered, no indication of the amount of crossing was obtained; other experiments are being carried on which will eventually clarify this point. Additional plants from the same sources were allowed to produce open-pollinated seed when planted together in the field in western Nebraska. Here again no information is yet available as to the exact amount of natural crossing. It is known, however, that some crossing occurred.

The results of the wilt-resistance tests conducted upon three such composites are given in Table 14. The three separate groups were made up as follows: No. 1 was composed of 9 plants from open-pollinated seed of plants from four old Nebraska fields which had come through one cold and two wilt tests. Similarly composite No. 2 was made up of 16 surviving plants from open-pollinated seed of plants from ten old Nebraska

TABLE 12.—*Cold resistance of alfalfa lines as affected by selfing and method of elimination* [1]

Family	Relative cold resistance in generations as indicated (Turkistan=100)					
	Open-pollinated	S_1	S_2	S_3	S_4	S_5
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Hardistan	100	102 CW 84 CW				
1-01	99	— CW	147 CW	139 CW 48 CW	107 W	
2-01	107	—	—	47 CW		
3-01	110	— —	— 140 C 136 W 97 CW	87 W 51 C 48 C		
3-02	126	93 CW 129 CW — 116 W	127 W 159 W — 66 C	97 W 36 C 73 CW	36 C 57 C 33 C 101 C	70 CW 110 CW 151 CW 85 CW
3-03	125	—	—	106 C		
3-05	104	87 C W	88 CW 85 W	150 CW		
3-06	109	117 W				
4-01	111	114 W 107 W 79 W 102 CW	50 C 100 C 109 C	144 CW		
4-02	105	61 W				
4-03	75	98 CW				
4-04	112	— 50 W 94 C	120 C 42 W 88 CW	115 CW		
4-06	73	—	—	128 W		
4-07	84	94 C	68 CW			
4-08	67	85 C	100 CW			
5-01	107	— 110 W 92 W	121 CW 128 C 82 C 121 C 75 C 122 C	54 C 121 W		
5-02	108	—	—	93 W	108 W	
5-03	100	21 W 143 W				
5-07	85	—	—	120 W 112 W 109 W 104 W 126 W	58 C 89 C 137 C 111 CW 90 C 96 W	167 CW 100 CW 79 W 65 W 48 W
5-08	77	67 CW				
6-01	121	— 61 C 117 C	43 C 72 C 196 C	63 CW		
7-01	94	— 90 CW	100 C 71 CW			

[1] The notations, C—cold, W—wilt, CW—cold and wilt, indicate the elimination test to which the immediate parent plant had been subjected and which it had survived.

TABLE 12.—*Cold resistance of alfalfa lines as affected by selfing and method of elimination* [1]*—(Continued)*

Family	Relative cold resistance in generations as indicated (Turkistan=100)					
	Open-pollinated	S_1	S_2	S_3	S_4	S_5
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
8-01	107	111 CW				
9-01	70	———	———	108 W		
		94 W				
		53 CW				
		———	45 W			
9-02	78	86 W				
		69 CW	76 CW			
		135 CW				
		76 CW				
9-03	109	48 W				
		107 CW				
Average	99	91	99	95	85	97

[1]The notations, C=cold, W=wilt, CW=cold and wilt, indicate the elimination test to which the immediate parent plant had been subjected and which it had survived.

fields. Composite No. 4 was composed of 16 plants from original seed lots of seven introduced Turkistans which had survived two wilt tests.

The average wilt resistance of the original seed varied from 53 per cent for composite No. 1 to 65 per cent for composite No. 4. The average wilt resistance of the greenhouse progeny after the compositing was considerably higher, averaging 75 to 80 per cent, while the progeny of composites Nos. 2 and 4, after crossing in the field, averaged 69 to 84 per cent.

From results already presented on selfing it would be expected that with the elimination treatment to which these plants were subjected, the progeny should at least maintain or even slightly increase their wilt resistance. The large increase in wilt resistance as here obtained may possibly be attributed to the effect of crossing.

In an experiment to determine the influence of cold elimination on cold resistance, a large number of plants of Turkistan F. C. I. No. 15754 were severely frozen and the survivors crossed in the greenhouse as described above. The resulting seed was then tested for cold resistance against the original. As an average of twelve replicates the original had 22 per cent of the plants surviving the test, while 40 per cent of the plants from the seed of the selected and crossed plants survived, thus indicating a decided increase in cold resistance.

DISCUSSION

The results presented dealing with the cold and wilt resistance of self-fertilized alfalfa have been largely confined to those families produced from old Nebraska fields, and very little data are included from the more recent Turkistan introductions. Data from the latter, however, have been analyzed for the first three generations and the results have conformed so nearly with those already given that it was deemed unnecessary to report them at this time.

TABLE 13.—*Summary of cold resistance of alfalfa lines as affected by selfing and methods of elimination*

Lines	Method of elimination			
	None	Wilt	Cold	Cold and wilt
Open-pollinated				
Number of lines	23	....	....	....
Percentage resistant	99	....	....	....
S_1 Number of lines	21	14	6	14
Percentage resistant	76	88	90	94
S_2 Number of lines	11	6	15	9
Percentage resistant	78	99	102	95
S_3 Number of lines	4	11	5	8
Percentage resistant	37	110	59	97
S_4 Number of lines	2	3	8	1
Percentage resistant	44	104	75	111
S_5 Number of lines	....	3	....	6
Percentage resistant	....	64	....	114
Weighted average				
Number of lines	38	37	34	38
Percentage resistant	71	96	87	98

Certain other varieties such as Ladak and Grimm have also been used for foundation material. As previously noted, Grimm has proved completely susceptible. Ladak has, however, proved more complex. Since the variety Ladak has many desirable qualities, a special effort has been made to secure resistant inbred lines from this source. Unfortunately most attempts have ended in failure, as most self-fertilized lines have rapidly decreased in cold or wilt resistance, if not in the first generation then in advanced generations. There have been one or two Ladak lines, however, which have continued high in resistance and these are being continued

TABLE 14.—*Comparison of the wilt resistance of seedlings from original open-pollinated seed with those of three composites, the parent plants of which had survived the elimination tests*

Composite	Origin of material	Families involved	Plants involved	Average healthy of original seed	Subsequent elimination	Average healthy	
						After crossing in greenhouse [1]	After crossing in field [2]
No.		*No.*	*No.*	*Per ct.*		*Per cent*	*Per cent*
1	Nebraska (old fields)	4	9	53 (4)[3]	2 wilt and cold	79 (9)[3]	....
2	Nebraska (old fields)	10	16	63 (10)	2 wilt	80 (15)	69 (16)[3]
4	Turkistan	7	16	65 (16)	2 wilt	75 (13)	84 (16)

[1] Average per cent of survival of seedlings from seed of the individual plants.
[2] Average per cent of survival of seedlings from composited seed.
[3] Figure in parenthesis denotes number of trials.

in the hope that they will maintain their cold and wilt resistance. It is believed much of the reduction in resistance occurring in self-fertilized lines of Ladak can be traced to its hybrid origin, the variety itself being very heterozygous. It seems apparent that many of the Turkistan alfalfas are much more homozygous in various characters, including wilt resistance, than most of the domestic alfalfas now commonly grown.

No detailed studies have been made to determine the inheritance of cold and wilt resistance in alfalfa, chiefly because of the unknown nature of the population concerned. It has been of considerable interest, however, to classify all the self-fertilized lines with respect to wilt resistance, irrespective of generation and type of elimination and to note that in various populations a rather definite distribution has been found. Analysis of 481 selfed lines resulted in the distribution curve shown in Figure 3.

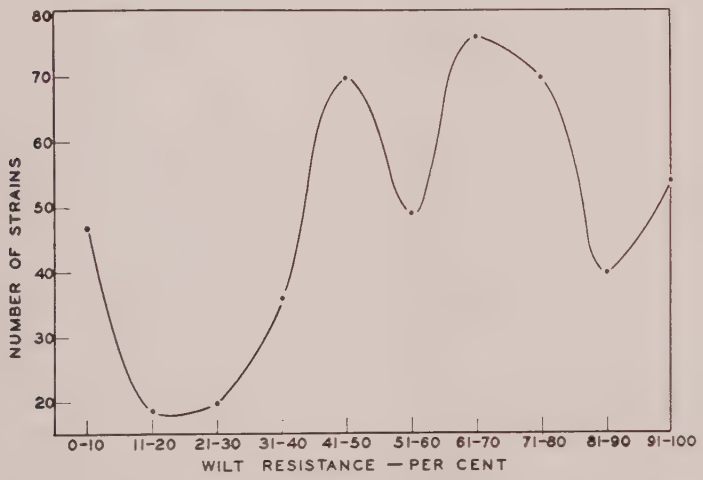

FIG. 3.—Classification of 481 selfed strains with respect to wilt resistance. The group includes Grimm, Ladak, Persian, and Turkistan alfalfas.

There are four distinctly modal points in the distribution. The first one is from 0-10 per cent healthy, resulting from the high susceptibility of selections of common, Grimm, Ladak, and a few other sorts of alfalfa. The second mode occurs at about 40 to 50 per cent resistance. These include a large number of Turkistan and some Persian and Ladak selections. The third mode occurs at from 60 to 80 per cent resistance and includes for the most part the more resistant Turkistan selections, while the fourth mode, from 90 to 100 per cent, represents those few selections, mostly Turkistan, which are very high in wilt resistance.

Three major genetic factor pairs for resistance to wilt may explain the type of curve obtained. While the evidence is not sufficient to arrive at a

definite conclusion, various populations have been analyzed separately with rather close agreement.

The data on relative cold resistance were similarly analyzed, but no definite modal points appeared in the distribution. It appears that the inheritance of cold resistance is undoubtedly due to a number of factors, as no clear-cut segregation was obtained.

The tests indicate a very slight improvement in wilt resistance by cold elimination, and a rather more decided improvement in cold resistance by wilt elimination. Since all available data suggest independent segregation of these characters, these results may be explained on the premise that the tests serve to eliminate the weaker individuals with respect to either cold or wilt resistance. That it is possible to obtain a relatively large number of selections having cold and wilt resistance is shown by a correlation coefficient of $.4535 \pm .043$ as determined from 157 selections which had been tested for cold and wilt resistance.

[3½M]

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